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(71) Applicant: SMITHKLINE BEECHAM CORPORATION
Philadelphia Pennsylvania 19103 (US)

(72) Inventors:
- Sathe, Ganesh Madhusudan,
SmithKline Beecham Phar.
King of Prussia, Pennsylvania 19406 (US)
- Halsey, Wendy S. SmithKline Beecham Phar.
King of Prussia, Pennsylvania 19406 (US)

(74) Representative:
Connell, Anthony Christopher et al
SmithKline Beecham plc
Corporate Intellectual Property,
Two New Horizons Court
Brentford, Middlesex TW8 9EP (GB)

(54) Cdna clone HNEAA81 that encodes a human 7-transmembrane receptor

(57) HNEAA81 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing HNEAA81 polypeptides and polynucleotides in the design of protocols for the treatment of infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, among others and diagnostic assays for such conditions.

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been successfully introduced into the market.

[0011] This indicates that these receptors have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome.

SUMMARY OF THE INVENTION

[0012] In one aspect, the invention relates to HNEAA81 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such HNEAA81 polypeptides and polynucleotides. Such uses include the treatment of infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with HNEAA81 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate HNEAA81 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

[0013] The following definitions are provided to facilitate understanding of certain terms used frequently herein.

[0014] "HNEAA81" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, or an allelic variant thereof.

[0015] "Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said HNEAA81 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said HNEAA81.

[0016] "HNEAA81 gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

[0017] "Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

[0018] "Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

[0019] "Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single-and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

[0020] "Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres.

[0021] "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and

to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross(inks, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NYAcad Sci* (1992) 663:48-62.

[0022] "Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

[0023] "Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al., Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al., J Molec Biol* (1990) 215:403).

[0024] As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: I is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference

sequence or in one or more contiguous groups within the reference sequence.

[0025] Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% identity to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

[0026] In one aspect, the present invention relates to HNEAA81 polypeptides (or HNEAA81 proteins). The HNEAA81 polypeptides include the polypeptide of SEQ ID NO:2; as well as polypeptides comprising the amino acid sequence of SEQ ID NO:2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within HNEAA81 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO: 2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Preferably, HNEAA81 polypeptides exhibit at least one biological activity of the receptor.

[0027] The HNEAA81 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

[0028] Fragments of the HNEAA81 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned HNEAA81 polypeptides. As with HNEAA81 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of the HNEAA81 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

[0029] Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of HNEAA81 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

[0030] Preferably, all of these polypeptide fragments retain the biological activity of the receptor, including antigenic activity. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions - i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

[0031] The HNEAA81 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

[0032] Another aspect of the invention relates to HNEAA81 polynucleotides. HNEAA81 polynucleotides include isolated polynucleotides which encode the HNEAA81 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, the HNEAA81 polynucleotides of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding an HNEAA81 polypeptide of SEQ ID NO: 2, and polynucleotide having the particular sequence of SEQ ID NO:1. HNEAA81 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the HNEAA81 polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under HNEAA81 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO: 1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such HNEAA81 polynucleotides.

[0033] HNEAA81 of the invention is structurally related to other proteins of the G-protein coupled receptor family, as shown by the results of sequencing the cDNA of Table 1 (SEQ ID NO:1) encoding human HNEAA81. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 98 to 1096) encoding a polypeptide of 333 amino acids (SEQ ID NO:2). The amino acid sequence of Table 2 (SEQ ID NO:2) has about 74.914 % identity in 293 amino acid residues with human G-protein coupled receptor; GPR3 (Geneseqp patent database, Accession # W04246, Bult, C.J. et al, 13 Dec. 1996). Furthermore, HNEAA81 (SEQ ID NO:2) is 28.0 % identical (FASTA, Swisspro databse) to platelet activating factor receptor over 293 amino acid residues (Honda, Z.I. et al, Nature, 349:342-346, 1991). Furthermore, HNEAA81 (SEQ ID No:2) is 25.6 % identical to thrombin receptor over 305 amino acid residues (Accession # P47749, Turck, C.W.et al, Nature, 368: 648-651, 1994). Furthermore, HNEAA81 (SEQ ID NO:2) is 26.5 % identical to EBV-Induced G-protein coupled receptor, EBI2 over 313 amino acid residues (Accession # P32249, Elliott, K et al, J. Virol. 67: 2209-2220, 1993). The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 96 % identity in 1124 nucleotide residues with human G-protein coupled receptor (Geneseqn patent database, Accession # T33904, Bult,C.J. et al, 13 Dec. 1996). Furthermore, HNEAA81 (SEQ ID No: 1) is 56.47 % identical (BLAST using Genebank database) to human mRNA for KIA0001 gene over 850 nucleotide residues (Accession # D13626, Nomura, N. et al, Unpublished, 1994). Thus, HNEAA81 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Table 1[a]

```
   1  TCTGGTTTTT AAAAAATAGC ATTTGAAAAT CATGAAGGGC TTTTTGTTTT

  51  CTTTTGTTTG TATATATGTT TATTGGTAAC AGGTGACACT GGAAGCAATG

 101  AACACCACAG TGATGCAAGG CTTCAACAGA TCTGAGCGGT GCCCCAGAGA

 151  CACTCGGATA GTACAGCTGG TATTCCCAGC CCTCTACACA GTGGTTTTCT

 201  TGACCGGCAT CCTGCTGAAT ACTTTGGCTC TGTGGGTGTT TGTTCACATC

 251  CCCAGCTCCT CCACCTTCAT CATCTACCTC AAAAACACTT TGGTGGCCGA

 301  CTTGATAATG ACACTCATGC TTCCTTTCAA AATCCTCTCT GACTCACACC

 351  TGGCACCCTG GCAGCTCAGA GCTTTTGTGT GTCGTTTTTC TTCGGTGATA

 401  TTTTATGAGA CCATGTATGT GGGCATCGTG CTGTTAGGGC TCATAGCCTT

 451  TGACAGATTC CTCAAGATCA TCAGACCTTT GAGAAATATT TTTCTAAAAA

 501  AACCTGTTTT TGCAAAAACG GTCTCAATCT TCATCTGGTT CTTTTTGTTC

 551  TTCATCTCCC TGCCAAATAC GATCTTGAGC AACAAGGAAG CAACACCATC

 601  GTCTGTGAAA AAGTGTGCTT CCTTAAAGGG GCCTCTGGGG CTGAAATGGC

 651  ATCAAATGGT AAATAACATA TGCCAGTTTA TTTTCTGGAC TGTTTTTATC

 701  CTAATGCTTG TGTTTTATGT GGTTATTGCA AAAAAAGTAT ATGATTCTTA

 751  TAGAAAGTCC AAAAGTAAGG ACAGAAAAAA CAACAAAAAG CTGGAAGGCA

 801  AAGTATTTGT TGTCGTGGCT GTCTTCTTTG TGTGTTTTGC TCCATTTCAT

 851  TTTGCCAGAG TTCCATATAC TCACAGTCAA ACCAACAATA AGACTGACTG

 901  TAGACTGCAA AATCAACTGT TTATTGCTAA AGAAACAACT CTCTTTTTGG

 951  CAGCAACTAA CATTTGTATG GATCCCTTAA TATACATATT CTTATGTAAA

1001  AAATTCACAG AAAAGCTACC ATGTATGCAA GGGAGAAAGA CCACAGCATC

1051  AAGCCAAGAA AATCATAGCA GTCAGACAGA CAACATAACC TTAGGCTGAC

1101  AACTGTACAT AGGGTTAACT TCTA
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[a] A nucleotide sequence of a human HNEAA81 (SEQ ID NO: 1).

Table 2[b]

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  1  MNTTVMQGFN RSERCPRDTR IVQLVFPALY TVVFLTGILL NTLALWVFVH

 51  IPSSSTFIIY LKNTLVADLI MTLMLPFKIL SDSHLAPWQL RAFVCRFSSV

101  IFYETMYVGI VLLGLIAFDR FLKIIRPLRN IFLKKPVFAK TVSIFIWFFL

151  FFISLPNTIL SNKEATPSSV KKCASLKGPL GLKWHQMVNN ICQFIFWTVF

201  ILMLVFYVVI AKKVYDSYRK SKSKDRKNNK KLEGKVFVVV AVFFVCFAPF

251  HFARVPYTHS QTNNKTDCRL QNQLFIAKET TLFLAATNIC MDPLIYIFLC

301  KKFTEKLPCM QGRKTTASSQ ENHSSQTDNI TLG
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[b] An amino acid sequence of a human HNEAA81 (SEQ ID NO: 2).

[0034] One polynucleotide of the present invention encoding HNEAA81 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human brain, Leukocyte, and lung using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature,* (1992) *355*:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

[0035] The nucleotide sequence encoding the HNEAA81 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 98 to 1096 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

[0036] When the polynucleotides of the invention are used for the recombinant production of an HNEAA81 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al., Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain noncoding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

[0037] Further preferred embodiments are polynucleotides encoding HNEAA81 variants comprising the amino acid sequence of the HNEAA81 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

[0038] The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

[0039] Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding HNEAA81 and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the HNEAA81 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide

sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

[0040] In one embodiment, to obtain a polynucleotide encoding the HNEAA81 polypeptide, including homologs and orthologs from species other than human, the method comprises screening an appropriate library under stingent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Thus in another aspect, HNEAA81 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof. Also included with HNEAA81 polypeptides are polypeptides comprising amino acid sequences encoded by nucleotide sequences obtained by the above hybridization condition. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or, alternatively, conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

[0041] The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

[0042] The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

[0043] For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al, *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAF-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

[0044] Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli, Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

[0045] A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al., MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

[0046] For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

[0047] If the HNEAA81 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If the HNEAA81 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

[0048] HNEAA81 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

[0049] This invention also relates to the use of HNEAA81 polynucleotides for use as diagnostic reagents. Detection of a mutated form of the HNELAA81 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of HNEAA81. Individuals carrying mutations in the HNEAA81 gene may be detected at the DNA level by a variety of techniques.

[0050] Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled HNEAA81 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al., Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al., Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotide probes comprising the HNEAA81 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

[0051] The diagnostic assays offer a process for diagnosing or determining a susceptibility to infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, through detection of mutation in the HNEAA81 gene by the methods described.

[0052] In addition, infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of the HNEAA81 polypeptide or HNEAA81 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an HNEAA81, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

[0053] Thus in another aspect, the present invention relates to a diagonostic kit for a disease or suspectability to a disease, particularly infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, which comprises:

(a) an HNEAA81 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof;
(b) a nucleotide sequence complementary to that of (a);
(c) an HNEAA81 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or
(d) an antibody to an HNEAA81 polypeptide, preferably to the polypeptide of SEQ ID NO: 2. It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Chromosome Assays

[0054] The nucleotide sequences of the present invention are also valuable for chromosome identification. The se-

quence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

[0055] The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the HNEAA81 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

[0056] Antibodies generated against the HNEAA81 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al., Immunology Todqy* (1983)4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

[0057] Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

[0058] The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

[0059] Antibodies against HNEAA81 polypeptides may also be employed to treat infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, among others.

Vaccines

[0060] Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with the HNEAA81 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises delivering the HNEAA81 polypeptide via a vector directing expression of the HNEAA81 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

[0061] A further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to an HNEAA81 polypeptide wherein the composition comprises an HNEAA81 polypeptide or HNEAA81 gene. The vaccine formulation may further comprise a suitable carrier. Since HNEAA81 polypeptides may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may

be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

[0062] The HNEAA81 polypeptide of the present invention may be employed in a screening process for compounds which bind the receptor and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See Coligan *et al., Current Protocols in Immunology* 1(2):Chapter 5 (1991).

[0063] HNEAA81 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate HNEAA81 on the one hand and which can inhibit the function of HNEAA81 on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome.

[0064] In general, such screening procedures involve producing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response.

[0065] One screening technique includes the use of cells which express receptors of this invention (for example, transfected CHO cells) in a system which measures extracellular pH or intracellular calcium changes caused by receptor activation. In this technique, compounds may be contacted with cells expressing the receptor polypeptide of the present invention. A second messenger response, e.g., signal transduction, pH changes, or changes in calcium level, is then measured to determine whether the potential compound activates or inhibits the receptor.

[0066] Another method involves screening for receptor inhibitors by determining inhibition or stimulation of receptor-mediated cAMP and/or adenylate cyclase accumulation. Such a method involves transfecting a eukaryotic cell with the receptor of this invention to express the receptor on the cell surface. The cell is then exposed to potential antagonists in the presence of the receptor of this invention. The amount of cAMP accumulation is then measured. If the potential antagonist binds the receptor, and thus inhibits receptor binding, the levels of receptor-mediated cAMP, or adenylate cyclase, activity will be reduced or increased.

[0067] Another method for detecting agonists or antagonists for the receptor of the present invention is the yeast based technology as described in U.S. Patent No. 5,482,835, incorporated by reference herein.

[0068] The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the receptor is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the receptor, using detection systems appropriate to the cells bearing the receptor at their surfaces. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

[0069] Further, the assays may simply comprise the steps of mixing a candidate compound with a solution containing an HNEAA81 polypeptide to form a mixture, measuring HNEAA81 activity in the mixture, and comparing the HNEAA81 activity of the mixture to a standard.

[0070] The HNEAA81 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of HNEAA81 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of HNEAA81 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of HNEAA81 (also called antagonist or agonist, respectively) from suitably manipulated

cells or tissues. Standard methods for conducting screening assays are well understood in the art.

[0071] Examples of potential HNEAA81 antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligand of the HNEAA81, e.g., a fragment of the ligand, or small molecules which bind to the receptor but do not elicit a response, so that the activity of the receptor is prevented.

[0072] Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for HNEAA81 polypeptides; or compounds which decrease or enhance the production of HNEAA81 polypeptides, which comprises:

(a) an HNEAA81 polypeptide, preferably that of SEQ ID NO:2;
(b) a recombinant cell expressing an HNEAA81 polypeptide, preferably that of SEQ ID NO:2;
(c) a cell membrane expressing an HNEAA81 polypeptide; preferably that of SEQ ID NO: 2; or
(d) antibody to an HNEAA81 polypeptide, preferably that of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Prophylactic and Therapeutic Methods

[0073] This invention provides methods of treating abnormal conditions such as, infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, related to both an excess of, and insufficient amounts of, HNEAA81 activity.

[0074] If the activity of HNEAA81 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the HNEAA81, or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of HNEAA81 polypeptides still capable of binding the ligand in competition with endogenous HNEAA81 may be administered. Typical embodiments of such competitors comprise fragments of the HNEAA81 polypeptide.

[0075] In still another approach, expression of the gene encoding endogenous HNEAA81 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al., Nucleic Acids Res* (1979)6:3073; *Cooney et al., Science* (1988)241:456; Dervan *et al., Science* (1991)251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

[0076] For treating abnormal conditions related to an under-expression of HNEAA81 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates HNEAA81, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of HNEAA81 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches,* (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of HNEAA81 polypeptides in combination with a suitable pharmaceutical carrier.

Formulation and Administration

[0077] Peptides, such as the soluble form of HNEAA81 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention

further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

[0078] Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

[0079] Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

[0080] The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

[0081] Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Example 1: Mammalian Cell Expression

[0082] The receptors of the present invention are expressed in either human embryonic kidney 293 (HEK293) cells or adherent dhfr CHO cells. To maximize receptor expression, typically all 5' and 3' untranslated regions (UTRs) are removed from the receptor cDNA prior to insertion into a pCDN or pCDNA3 vector. The cells are transfected with individual receptor cDNAs by lipofectin and selected in the presence of 400 mg/ml G418. After 3 weeks of selection, individual clones are picked and expanded for further analysis. HEK293 or CHO cells transfected with the vector alone serve as negative controls. To isolate cell lines stably expressing the individual receptors, about 24 clones are typically selected and analyzed by Northern blot analysis. Receptor mRNAs are generally detectable in about 50% of the G418-resistant clones analyzed.

Example 2 Ligand bank for binding and functional assays.

[0083] A bank of over 200 putative receptor ligands has been assembled for screening. The bank comprises: transmitters, hormones and chemokines known to act via a human seven transmembrane (7TM) receptor; naturally occurring compounds which may be putative agonists for a human 7TM receptor, non-mammalian, biologically active peptides for which a mammalian counterpart has not yet been identified; and compounds not found in nature, but which activate 7TM receptors with unknown natural ligands. This bank is used to initially screen the receptor for known ligands, using both functional (i.e . calcium, cAMP, microphysiometer, oocyte electrophysiology, etc, see below) as well as binding assays.

Example 3: Ligand Binding Assays

[0084] Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for a receptor is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards its receptor. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell receptor sources. For these assays, specific receptor binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

Example 4: Functional Assay in Xenopus Oocytes

[0085] Capped RNA transcripts from linearized plasmid templates encoding the receptor cDNAs of the invention are synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended

in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual Xenopus oocytes in response to agonist exposure. Recordings are made in Ca2+ free Barth's medium at room temperature. The Xenopus system can be used to screen known ligands and tissue/cell extracts for activating ligands.

Example 5: Microphysiometric Assays

[0086] Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, CA). The CYTOSENSOR is thus capable of detecting the activation of a receptor which is coupled to an energy utilizing intracellular signaling pathway such as the G-protein coupled receptor of the present invention.

Example 6: Extract/Cell Supernatant Screening

[0087] A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligand banks as identified to date. Accordingly, the 7TM receptor of the invention is also functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts to identify natural ligands. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

Example 7: Calcium and cAMP Functional Assays

[0088] 7TM receptors which are expressed in HEK 293 cells have been shown to be coupled functionally to activation of PLC and calcium mobilization and/or cAMP stimuation or inhibition. Basal calcium levels in the HEK 293 cells in receptor-transfected or vector control cells were observed to be in the normal, 100 nM to 200 nM, range. HEK 293 cells expressing recombinant receptors are loaded with fura 2 and in a single day greater than 150 selected ligands or tissue/cell extracts are evaluated for agonist induced calcium mobilization. Similarly, HEK 293 cells expressing recombinant receptors are evaluated for the stimulation or inhibition of cAMP production using standard cAMP quantitation assays. Agonists presenting a calcium transient or cAMP fluctuation are tested in vector control cells to determine if the response is unique to the transfected cells expressing receptor.

[0089] All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

Annex to the description

[0090]

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT
(A) NAME: SmithKline Beecham Corporation
(B) STREET: One Franklin Plaza
(C) CITY: Philadelphia
(D) STATE OR PROVINCE: Pennsylvania
(E) COUNTRY: USA
(F) POSTAL CODE: 19103

(ii) TITLE OF THE INVENTION: cDNA CLONE HNEAA81 THAT ENCODES A HUMAN 7-TRANSMEMBRANE RECEPTOR

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER-READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1124 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCTGGTTTTT AAAAAATAGC ATTTGAAAAT CATGAAGGGC TTTTTGTTTT CTTTTGTTTG 60
TATATATGTT TATTGGTAAC AGGTGACACT GGAAGCAATG AACACCACAG TGATGCAAGG 120
CTTCAACAGA TCTGAGCGGT GCCCCAGAGA CACTCGGATA GTACAGCTGG TATTCCCAGC 180

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CCTCTACACA GTGGTTTTCT TGACCGGCAT CCTGCTGAAT ACTTTGGCTC TGTGGGTGTT    240
TGTTCACATC CCCAGCTCCT CCACCTTCAT CATCTACCTC AAAAACACTT TGGTGGCCGA    300
CTTGATAATG ACACTCATGC TTCCTTTCAA AATCCTCTCT GACTCACACC TGGCACCCTG    360
GCAGCTCAGA GCTTTTGTGT GTCGTTTTTC TTCGGTGATA TTTTATGAGA CCATGTATGT    420
GGGCATCGTG CTGTTAGGGC TCATAGCCTT TGACAGATTC CTCAAGATCA TCAGACCTTT    480
GAGAAATATT TTTCTAAAAA AACCTGTTTT TGCAAAAACG GTCTCAATCT TCATCTGGTT    540
CTTTTTGTTC TTCATCTCCC TGCCAAATAC GATCTTGAGC AACAAGGAAG CAACACCATC    600
GTCTGTGAAA AAGTGTGCTT CCTTAAAGGG GCCTCTGGGG CTGAAATGGC ATCAAATGGT    660
AAATAACATA TGCCAGTTTA TTTTCTGGAC TGTTTTTATC CTAATGCTTG TGTTTTATGT    720
GGTTATTGCA AAAAAAGTAT ATGATTCTTA TAGAAAGTCC AAAAGTAAGG ACAGAAAAAA    780
CAACAAAAAG CTGGAAGGCA AAGTATTTGT TGTCGTGGCT GTCTTCTTTG TGTGTTTTGC    840
TCCATTTCAT TTTGCCAGAG TTCCATATAC TCACAGTCAA ACCAACAATA AGACTGACTG    900
TAGACTGCAA AATCAACTGT TTATTGCTAA AGAAACAACT CTCTTTTTGG CAGCAACTAA    960
CATTTGTATG GATCCCTTAA TATACATATT CTTATGTAAA AAATTCACAG AAAAGCTACC   1020
ATGTATGCAA GGGAGAAAGA CCACAGCATC AAGCCAAGAA AATCATAGCA GTCAGACAGA   1080
CAACATAACC TTAGGCTGAC AACTGTACAT AGGGTTAACT TCTA                    1124
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 333 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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 1               5                  10                  15
Arg Asp Thr Arg Ile Val Gln Leu Val Phe Pro Ala Leu Tyr Thr Val
            20                  25                  30
Val Phe Leu Thr Gly Ile Leu Leu Asn Thr Leu Ala Leu Trp Val Phe
        35                  40                  45
Val His Ile Pro Ser Ser Ser Thr Phe Ile Ile Tyr Leu Lys Asn Thr
    50                  55                  60
Leu Val Ala Asp Leu Ile Met Thr Leu Met Leu Pro Phe Lys Ile Leu
65                  70                  75                  80
Ser Asp Ser His Leu Ala Pro Trp Gln Leu Arg Ala Phe Val Cys Arg
                85                  90                  95
Phe Ser Ser Val Ile Phe Tyr Glu Thr Met Tyr Val Gly Ile Val Leu
            100                 105                 110
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Leu Gly Leu Ile Ala Phe Asp Arg Phe Leu Lys Ile Ile Arg Pro Leu
        115                 120                 125
Arg Asn Ile Phe Leu Lys Lys Pro Val Phe Ala Lys Thr Val Ser Ile
    130                 135                 140
Phe Ile Trp Phe Phe Leu Phe Phe Ile Ser Leu Pro Asn Thr Ile Leu
145                 150                 155                 160
Ser Asn Lys Glu Ala Thr Pro Ser Ser Val Lys Lys Cys Ala Ser Leu
                165                 170                 175
Lys Gly Pro Leu Gly Leu Lys Trp His Gln Met Val Asn Asn Ile Cys
            180                 185                 190
Gln Phe Ile Phe Trp Thr Val Phe Ile Leu Met Leu Val Phe Tyr Val
        195                 200                 205
Val Ile Ala Lys Lys Val Tyr Asp Ser Tyr Arg Lys Ser Lys Ser Lys
    210                 215                 220
Asp Arg Lys Asn Asn Lys Lys Leu Glu Gly Lys Val Phe Val Val Val
225                 230                 235                 240
Ala Val Phe Phe Val Cys Phe Ala Pro Phe His Phe Ala Arg Val Pro
                245                 250                 255
Tyr Thr His Ser Gln Thr Asn Asn Lys Thr Asp Cys Arg Leu Gln Asn
            260                 265                 270
Gln Leu Phe Ile Ala Lys Glu Thr Thr Leu Phe Leu Ala Ala Thr Asn
        275                 280                 285
Ile Cys Met Asp Pro Leu Ile Tyr Ile Phe Leu Cys Lys Lys Phe Thr
    290                 295                 300
Glu Lys Leu Pro Cys Met Gln Gly Arg Lys Thr Thr Ala Ser Ser Gln
305                 310                 315                 320
Glu Asn His Ser Ser Gln Thr Asp Asn Ile Thr Leu Gly
                325                 330
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Claims

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 97% identity over its entire length to a nucleotide sequence encoding the HNEAA81 polypeptide of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.

2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO:1 encoding the HNEAA81 polypeptide of SEQ ID NO2.

3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 97% identical to that of SEQ ID NO: 1 over its entire length.

4. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.

5. The polynucleotide of claim 1 which is DNA or RNA.

6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing an HNEAA81 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide

of SEQ ID NO:2 when said expression system is present in a compatible host cell.

7. A host cell comprising the expression system of claim 6.

8. A process for producing an HNEAA81 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

9. A process for producing a cell which produces an HNEAA81 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces an HNEAA81 polypeptide.

10. An HNEAA81 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.

12. An antibody immunospecific for the HNEAA81 polypeptide of claim 10.

13. A method for the treatment of a subject in need of enhanced activity or expression of the HNEAA81 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an agonist to said receptor; and/or
(b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 97% identity to a nucleotide sequence encoding the HNEAA81 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.

14. A method for the treatment of a subject having need to inhibit activity or expression of the HNEAA81 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said receptor; and/or
(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said receptor; and/or
(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said receptor for its ligand.

15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of the HNEAA81 polypeptide of claim 10 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said HNEAA81 polypeptide in the genome of said subject; and/or
(b) analyzing for the presence or amount of the HNEAA81 polypeptide expression in a sample derived from said subject.

16. A method for identifying agonists to the HNEAA81 polypeptide of claim 10 comprising:

(a) contacting a cell which produces a HNEAA81 polypeptide with a candidate compound; and
(b) determining whether the candidate compound effects a signal generated by activation of the HNEAA81 polypeptide.

17. An agonist identified by the method of claim 16.

18. A method for identifying antagonists to the HNEAA81 polypeptide of claim 10 comprising:

(a) contacting a cell which produces an HNEAA81 polypeptide with an agonist; and
(b) determining whether the signal generated by said agonist is diminished in the presence of a candidate compound.

19. An antagonist identified by the method of claim 18.

20. A recombinant host cell produced by a method of Claim 9 or a membrane thereof expressing an HNEAA81 polypeptide.

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(71) Applicant *(for all designated States except US)*: INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: AU-YOUNG, Janice [US/US]; 1419 Kains Avenue, Berkeley, CA 94702 (US). GUEGLER, Karl, J. [CH/US]; 1048 Oakland Avenue, Menlo Park, CA 94025 (US). CHENG, Muzong [US/US]; 648 Alcatraz Avenue #301, Oakland, CA 94609 (US).

(74) Agent: BILLINGS, Lucy, J.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US).

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(57) Abstract

The present invention provides a human chemokine receptor-like protein (NHCR) and polynucleotides which identify and encode NHCR. The invention also provides expression vectors, host cells, agonists, antibodies, and antagonists. The invention also provides methods for treating disorders associated with expression of NHCR.

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HUMAN CHEMOKINE RECEPTOR-LIKE CHEMOKINE

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of a novel human chemokine receptor-like protein and to the use of these sequences in the diagnosis, prevention, and treatment of disorders associated with inflammation and viral infection.

BACKGROUND ART

Chemokines are a large family of low molecular weight, inducible, secreted, proinflammitory cytokines which are produced by various cell types. They have been divided into several subfamilies on the basis of the positions of their conserved cysteines. The CXC family includes interleukin-8 (IL-8), growth regulatory gene, neutrophil-activating peptide-2, and platelet factor 4 (PF-4). Although IL-8 and PF-4 are both polymorphonuclear chemoattractants, angiogenesis is stimulated by IL-8 and inhibited by PF-4. The CC family includes monocyte chemoattractant protein-1 (MCP-1), RANTES (regulated on activation, normal T cell-expressed and secreted), macrophage inflammatory proteins (MIP-1α, MIP-1β), and eotaxin. MCP-1 is secreted by numerous cell types including endothelial, epithelial, and hematopoietic cells, and is a chemoattractant for monocytes and CD45RO+ lymphocytes (Proost, P. (1996) Int J. Clin. Lab. Res. 26:211-223; Raport, C. J. (1996) J. Biol. Chem. 271:17161-17166).

Cells respond to chemokines through G-protein-coupled receptors. These receptors are seven transmembrane molecules which transduce their signal through heterotrimeric GTP-binding proteins. Stimulation of the GTP-binding protein complex by activated receptor leads to the exchange of guanosine diphosphate for guanosine triphosphate and regulates the activity of effector molecules. There are distinct classes of each of the subunits which differ in activity and specificity and can elicit inhibitory or stimulatory responses. When stimulation of the known cytokine receptors shows agonist-dependent inhibition of adenylyl cyclase and mobilization of intracellular calcium, the receptor coupling to $G_{\alpha}i$ subunits (Myers, S. J. et al (1995) J. Biol. Chem. 270:5786-5792).

Chemokine receptors play a major role in the mobilization and activation of cells of the immune system. The effects of receptor stimulation are dependent on the cell type and include chemotaxis, proliferation, differentiation, and production of cytokines. Chemokine stimulation produces changes in vascular endothelium, chemotaxis to sites of inflammation, and activates the effector functions of cells (Taub, D. D. (1996) Cytokine Growth Factor Rev. 7:355-376).

The chemokine receptors display a range of sequence diversity and ligand promiscuity. The known chemokine receptor protein sequence identities range from 22 to 40%, and certain

receptors can respond to multiple ligands. Although mainly expressed in immune cells, viral homologues are expressed by human cytomegalovirus and Herpesvirus saimiri. The chemokine receptor known as the Duffy blood group antigen binds both CC and CXC family chemokines and serves as the receptor on erythrocytes for the malarial parasite Plasmodium vivax. Members of the chemokine receptor family are used as co-receptors with CD4 for HIV-1 entry into target cells. Several receptors have recently been cloned. The human chemokine receptor, R12, was isolated by cross-hybridization of an APJ/R20 probe on a human genomic library. R12 is most similar to the R20 orphan receptor (which has homology with the angiotensin receptor) and shows between 22 and 26% homology to characterized chemokine receptors including IL-8A and B, and MCP-1α and 1β. (Murphy, P.M. (1994) Annu. Rev. Immunol. 12:593-633; Raport, C.J. et al (1996) J. Leuk. Biol. 59:18-23; He, J. et al (1997) Nature 385:645-649).

The discovery of proteins related to human chemokine receptor R12 and the polynucleotides encoding them satisfies a need in the art by providing new compositions useful in diagnosis and treatment of disorders associated with inflammation and viral infection.

DISCLOSURE OF THE INVENTION

The present invention features a novel human chemokine receptor-like protein hereinafter designated NHCR and characterized as having similarity to human chemokine receptor R12.

Accordingly, the invention features a substantially purified NHCR having the amino acid sequence shown in SEQ ID NO:1.

One aspect of the invention features isolated and substantially purified polynucleotides that encode NHCR. In a particular aspect, the polynucleotide is the nucleotide sequence of SEQ ID NO:2.

The invention also relates to a polynucleotide sequence comprising the complement of SEQ ID NO:2 or variants thereof. In addition, the invention features polynucleotide sequences which hybridize under stringent conditions to SEQ ID NO:2.

The invention additionally features nucleic acid sequences encoding polypeptides, oligonucleotides, peptide nucleic acids (PNA), fragments, portions or antisense molecules thereof, and expression vectors and host cells comprising polynucleotides that encode NHCR. The present invention also features antibodies which bind specifically to NHCR, and pharmaceutical compositions comprising substantially purified NHCR. The invention also features the use of agonists and antagonists of NHCR. The invention also features a method for treating disorders associated with decreased NHCR by administering NHCR and a method for

treating disorders associated with increased NHCR by administering an antagonist to NHCR.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1A, 1B, 1C, 1D and 1E show the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:2) of NHCR. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co., Ltd., San Bruno, CA).

Figures 2A and 2B shows the amino acid sequence alignment between NHCR (SEQ ID NO:1) and a human chemokine receptor (GI 992700; SEQ ID NO:3). The alignment was produced using the multisequence alignment program of DNASTAR™ software (DNASTAR Inc, Madison WI).

Figures 3A and 3B show the hydrophobicity plots (MacDNASIS PRO software) for NHCR and human chemokine receptor, respectively; the positive X axis reflects amino acid position, and the negative Y axis, hydrophobicity.

MODES FOR CARRYING OUT THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a host cell" includes a plurality of such host cells, reference to the "antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"Nucleic acid sequence" as used herein refers to an oligonucleotide, nucleotide, or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand. Similarly, "amino acid sequence" as used herein refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragments or portions thereof, and to naturally occurring or synthetic molecules.

Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

"Peptide nucleic acid", as used herein, refers to a molecule which comprises an oligomer to which an amino acid residue, such as lysine, and an amino group have been added. These small molecules, also designated anti-gene agents, stop transcript elongation by binding to their complementary strand of nucleic acid (Nielsen, P.E. et al. (1993) Anticancer Drug Des. 8:53-63).

NHCR, as used herein, refers to the amino acid sequences of substantially purified NHCR obtained from any species, particularly mammalian, including bovine, ovine, porcine, murine, equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant.

"Consensus", as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, or which has been extended using XL-PCR™ (Perkin Elmer, Norwalk, CT) in the 5' and/or the 3' direction and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte clone using the GELVIEW™ Fragment Assembly system (GCG, Madison, WI), or which has been both extended and assembled.

A "variant" of NHCR, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

A "deletion", as used herein, refers to a change in either amino acid or nucleotide sequence in which one or more amino acid or nucleotide residues, respectively, are absent.

An "insertion" or "addition", as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues, respectively, as compared to the naturally occurring molecule.

A "substitution", as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

The term "biologically active", as used herein, refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic NHCR, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "agonist", as used herein, refers to a molecule which, when bound to NHCR, causes a change in NHCR which modulates the activity of NHCR. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to NHCR.

The terms "antagonist" or "inhibitor", as used herein, refer to a molecule which, when bound to NHCR, blocks or modulates the biological or immunological activity of NHCR. Antagonists and inhibitors may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to NHCR.

The term "modulate", as used herein, refers to a change or an alteration in the biological activity of NHCR. Modulation may be an increase or a decrease in protein activity, a change in binding characteristics, or any other change in the biological, functional or immunological properties of NHCR.

The term "mimetic", as used herein, refers to a molecule, the structure of which is developed from knowledge of the structure of NHCR or portions thereof and, as such, is able to effect some or all of the actions of NHCR-like molecules.

The term "derivative", as used herein, refers to the chemical modification of a nucleic acid encoding NHCR or the encoded NHCR. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group. A nucleic acid derivative would encode a polypeptide which retains essential biological characteristics of the natural molecule.

The term "substantially purified", as used herein, refers to nucleic or amino acid sequences that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which

they are naturally associated.

"Amplification" as used herein refers to the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction (PCR) technologies well known in the art (Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY).

The term "hybridization", as used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

The term "hybridization complex", as used herein, refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen binds between complementary G and C bases and between complementary A and T bases; these hydrogen bonds may be further stabilized by base stacking interactions. The two complementary nucleic acid sequences hydrogen bond in an antiparallel configuration. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., membranes, filters, chips, pins or glass slides to which cells have been fixed for in situ hybridization).

The terms "complementary" or "complementarity", as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, for the sequence "A-G-T" binds to the complementary sequence "T-C-A". Complementarity between two single-stranded molecules may be "partial", in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands.

The term "homology", as used herein, refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). A partially complementary sequence is one that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid; it is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous sequence or probe to the target sequence under conditions of low stringency. This is not to say that conditions of low

stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% identity); in the absence of non-specific binding, the probe will not hybridize to the second non-complementary target sequence.

As known in the art, numerous equivalent conditions may be employed to comprise either low or high stringency conditions. Factors such as the length and nature (DNA, RNA, base composition) of the sequence, nature of the target (DNA, RNA, base composition, presence in solution or immobilization, etc.), and the concentration of the salts and other components (e.g., the presence or absence of formamide, dextran sulfate and/or polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of either low or high stringency different from, but equivalent to, the above listed conditions.

The term "stringent conditions", as used herein, is the "stringency" which occurs within a range from about Tm-5°C (5°C below the melting temperature (Tm) of the probe) to about 20°C to 25°C below Tm. As will be understood by those of skill in the art, the stringency of hybridization may be altered in order to identify or detect identical or related polynucleotide sequences.

The term "antisense", as used herein, refers to nucleotide sequences which are complementary to a specific DNA or RNA sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules may be produced by any method, including synthesis by ligating the gene(s) of interest in a reverse orientation to a viral promoter which permits the synthesis of a complementary strand. Once introduced into a cell, this transcribed strand combines with natural sequences produced by the cell to form duplexes. These duplexes then block either the further transcription or translation. In this manner, mutant phenotypes may be generated. The designation "negative" is sometimes used in reference to the antisense strand, and "positive" is sometimes used in reference to the sense strand.

The term "portion", as used herein, with regard to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid. Thus, a protein "comprising at least a portion of the amino acid sequence of SEQ ID NO:1" encompasses the full-length human NHCR and fragments thereof.

"Transformation", as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. It may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

The term "antigenic determinant", as used herein, refers to that portion of a molecule that makes contact with a particular antibody (i.e., an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody and a protein or peptide, mean that the interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) on the protein; in other words, the antibody is recognizing and binding to a specific protein structure rather than to proteins in general. For example, if an antibody is specific for epitope "A", the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labeled "A" and the antibody will reduce the amount of labeled A bound to the antibody.

The term "sample", as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acid encoding NHCR or fragments thereof may comprise a cell, chromosomes isolated from a cell (e.g., a spread of metaphase chromosomes), genomic DNA (in solution or bound to a solid support such as for Southern analysis), RNA (in solution or bound to a solid support such as for northern analysis), cDNA (in solution or bound to a solid support), an extract from cells or a tissue, and the like.

The term "correlates with expression of a polynucleotide", as used herein, indicates that the detection of the presence of ribonucleic acid that is similar to SEQ ID NO:2 by northern analysis is indicative of the presence of mRNA encoding NHCR in a sample and thereby correlates with expression of the transcript from the polynucleotide encoding the protein.

"Alterations" in the polynucleotide of SEQ ID NO: 2, as used herein, comprise any alteration in the sequence of polynucleotides encoding NHCR including deletions, insertions, and point mutations that may be detected using hybridization assays. Included within this definition is the detection of alterations to the genomic DNA sequence which encodes NHCR (e.g., by alterations in the pattern of restriction fragment length polymorphisms capable of hybridizing to SEQ ID NO:2), the inability of a selected fragment of SEQ ID NO: 2 to hybridize to a sample of genomic DNA (e.g., using allele-specific oligonucleotide probes), and improper or unexpected hybridization, such as hybridization to a locus other than the normal chromosomal locus for the polynucleotide sequence encoding NHCR (e.g., using fluorescent in situ hybridization [FISH] to metaphase chromosomes spreads).

As used herein, the term "antibody" refers to intact molecules as well as fragments thereof, such as Fa, $F(ab')_2$, and Fv, which are capable of binding the epitopic determinant. Antibodies that bind NHCR polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or peptide used to immunize an animal can be derived from the transition of RNA or synthesized chemically, and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin and thyroglobulin. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

The term "humanized antibody", as used herein, refers to antibody molecules in which amino acids have been replaced in the non-antigen binding regions in order to more closely resemble a human antibody, while still retaining the original binding ability.

THE INVENTION

The invention is based on the discovery of a novel human chemokine receptor-like protein, (NHCR), the polynucleotides encoding NHCR, and the use of these compositions for the diagnosis, prevention, or treatment of disorders associated with inflammation and infection.

Nucleic acids encoding the human NHCR of the present invention were first identified in Incyte Clone 568987 from the macrophage cDNA library MMLR3DT01 through a computer-generated search for amino acid sequence alignments. The complete nucleotide sequence, SEQ ID NO:2, was derived from extension of Incyte clone 56897.

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1, as shown in Fig. 1. NHCR is 333 amino acids in length and has chemical and structural homology with human chemokine receptor (SEQ ID NO:3). In particular, NHCR and human chemokine receptor share 26% identity. Both NHCR and human

chemokine receptor contain a G-protein receptor motif, from V_{108} - I_{124} and A_{117} - I_{133}, respectively. In addition, NHCR and human chemokine receptor have potential amino terminal Asn-glycosylation sites, at N_{10} and at N_{14}, respectively, and a carboxy-terminal amidation site, at M_{310} and at L_{312}, respectively. As illustrated by Figs. 3A and 3B, NHCR and human cytokine receptor have rather similar hydrophobicity plots.

The invention also encompasses NHCR variants. A preferred NHCR variant is one having at least 80%, and more preferably 90%, amino acid sequence identity to the NHCR amino acid sequence (SEQ ID NO:1). A most preferred NHCR variant is one having at least 95% amino acid sequence identity to SEQ ID NO:1.

The invention also encompasses polynucleotides which encode NHCR. Accordingly, any nucleic acid sequence which encodes the amino acid sequence of NHCR can be used to generate recombinant molecules which express NHCR. In a particular embodiment, the invention encompasses the polynucleotide comprising the nucleic acid sequence of SEQ ID NO:2 as shown in Figures 1A, 1B, 1C, 1D and 1E.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding NHCR, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring NHCR, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode NHCR and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring NHCR under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding NHCR or its derivatives possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding NHCR and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences, or portions thereof,

which encode NHCR and its derivatives, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art at the time of the filing of this application. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding NHCR or any portion thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed nucleotide sequences, and in particular, those shown in SEQ ID NO:2, under various conditions of stringency. Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex or probe, as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol. 152:507-511), and may be used at a defined stringency.

Altered nucleic acid sequences encoding NHCR which are encompassed by the invention include deletions, insertions, or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent NHCR. The encoded protein may also contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent NHCR. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of NHCR is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid; positively charged amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; phenylalanine and tyrosine.

Also included within the scope of the present invention are alleles of the genes encoding NHCR. As used herein, an "allele" or "allelic sequence" is an alternative form of the gene which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

Methods for DNA sequencing which are well known and generally available in the art may be used to practice any embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp,

Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE Amplification System marketed by Gibco BRL (Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI 377 DNA sequencers (Perkin Elmer).

The nucleic acid sequences encoding NHCR may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed, "restriction-site" PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). In particular, genomic DNA is first amplified in the presence of primer to linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186). The primers may be designed using OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119). In this method, multiple restriction enzyme digestions and ligations may also be used to place an engineered double-stranded sequence into an unknown portion of the DNA molecule before performing PCR.

Another method which may be used to retrieve unknown sequences is that of Parker, J.D. et al. (1991; Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk in genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into the 5' and 3' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled devise camera. Output/light intensity may be converted to electrical signal using appropriate software (e.g. Genotyper™ and Sequence Navigator™, Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode NHCR, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of NHCR in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express NHCR.

As will be understood by those of skill in the art, it may be advantageous to produce NHCR-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter NHCR encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For

example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding NHCR may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of NHCR activity, it may be useful to encode a chimeric NHCR protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the NHCR encoding sequence and the heterologous protein sequence, so that NHCR may be cleaved and purified away from the heterologous moiety.

In another embodiment, sequences encoding NHCR may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of NHCR, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J.Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer).

The newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, supra). Additionally, the amino acid sequence of NHCR, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a biologically active NHCR, the nucleotide sequences encoding NHCR or functional equivalents, may be inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding NHCR and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press,

Plainview, NY, and Ausubel, F.M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY.

A variety of expression vector/host systems may be utilized to contain and express sequences encoding NHCR. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequences" are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, LaJolla, CA) or pSport1™ plasmid (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding NHCR, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for NHCR. For example, when large quantities of NHCR are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional E. coli cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence encoding NHCR may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of ß-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors (Promega, Madison, WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble

and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al. (1987) Methods Enzymol. 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding NHCR may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.

An insect system may also be used to express NHCR. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding NHCR may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of NHCR will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which NHCR may be expressed (Engelhard, E.K. et al. (1994) Proc. Nat. Acad. Sci. 91:3224-3227).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding NHCR may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing NHCR in infected host cells (Logan, J. and Shenk, T. (1984) Proc. Natl. Acad. Sci. 81:3655-3659). In addition, transcription

enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding NHCR. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding NHCR, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express NHCR may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al.

(1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1980) Cell 22:817-23) genes which can be employed in tk^- or $aprt^-$ cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, ß glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131). .

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding NHCR is inserted within a marker gene sequence, recombinant cells containing sequences encoding NHCR can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding NHCR under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain the nucleic acid sequence encoding NHCR and express NHCR may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

The presence of polynucleotide sequences encoding NHCR can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or portions or fragments of polynucleotides encoding NHCR. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding NHCR to detect transformants containing DNA or RNA encoding NHCR. As used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides,

which can be used as a probe or amplimer.

A variety of protocols for detecting and measuring the expression of NHCR, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on NHCR is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul, MN) and Maddox, D.E. et al. (1983; J. Exp. Med. 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding NHCR include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding NHCR, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp., Cleveland, OH). Suitable reporter molecules or labels, which may be used, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding NHCR may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode NHCR may be designed to contain signal sequences which direct secretion of NHCR through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding NHCR to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the

FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and NHCR may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing NHCR and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography as described in Porath, J. et al. (1992, Prot. Exp. Purif. 3: 263-281) while the enterokinase cleavage site provides a means for purifying NHCR from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. et al. (1993; DNA Cell Biol. 12:441-453).

In addition to recombinant production, fragments of NHCR may be produced by direct peptide synthesis using solid-phase techniques Merrifield J. (1963) J. Am. Chem. Soc. 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of NHCR may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

THERAPEUTICS

Based on the chemical and structural homology between NHCR (SEQ ID NO:1) and human chemokine receptor R12 (SEQ ID NO:3), NHCR appears to play a role in inflammation and viral infection.

Therefore, in one embodiment, antagonists or inhibitors of NHCR may be administered to a subject to prevent an inflammatory disorder associated with expression of NHCR. Types of inflammatory disorders may include, but are not limited to, allergic reactions, asthma and adult respiratory distress syndrome, rheumatoid arthritis, osteoarthritis, glomerulonephritis, osteoporosis, dermatomyositis, polymyositis, Addison's disease, Grave's disease, irritable bowel syndrome, atrophic gastritis, lupus erythematosus, myasthenia gravis, multiple sclerosis, autoimmune thyroiditis, ulcerative colitis, anemia, pancreatitis, scleroderma, Crohn's disease, ischemia/reperfusion injury, post-traumatic inflammation, myocardial inflammation, atherosclerosis, multiple sclerosis, and inflammatory complications of cancer, hemodialysis and extracorporeal circulation, infection and trauma. In one aspect, antibodies which are specific for NHCR may be used directly as an antagonist, or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express NHCR.

In another embodiment, a vector expressing the complement or antisense of the

polynucleotide encoding NHCR may be administered to a subject to treat or prevent the inflammatory disorders associated with NHCR expression listed above.

In another embodiment, antagonists or inhibitors of NHCR may be administered to a subject to prevent a viral infection which is associated with expression of NHCR. Types of viral infections may include, but are not limited to, HIV, Epstein-Barr virus, VZV, HTLV, CMV, bunyaviruses, influenza, adenovirus, rhinoviruses, hepadnaviruses, and rotaviruses.

In another embodiment, a vector expressing the complement or antisense of the polynucleotide encoding NHCR may be administered to a subject to prevent the viral infections associated with expression of NHCR listed above.

In another embodiment, NHCR or a fragment or derivative thereof may be administered to a subject to treat a disorder which is associated with reduced NHCR expression including, but not limited to, complement deficiency, immunodeficiency, sepsis, tumor growth, and impaired wound healing.

In another embodiment, a vector capable of expressing NHCR, or a fragment or a derivative thereof, may also be administered to a subject to treat a disorder associated with NHCR expression, and particularlythose listed in the preceeding paragraph.

In other embodiments, any of the therapeutic proteins, antagonists, antibodies, agonists, antisense sequences or vectors described above may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Antagonists or inhibitors of NHCR may be produced using methods which are generally known in the art. In particular, purified NHCR may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind NHCR.

The antibodies may be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies, (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with NHCR or any fragment or oligopeptide

thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the peptides, fragments, or oligopeptides used to induce antibodies to NHCR have an amino acid sequence consisting of at least five amino acids, and more preferably at least 10 amino acids. It is also preferable that they are identical to a portion of the amino acid sequence of the natural protein, and they may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of NHCR amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule.

Monoclonal antibodies to NHCR may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120).

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; Takeda, S. et al. (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce NHCR-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:11120-3).

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; Winter, G. et al. (1991) Nature 349:293-299).

Antibody fragments which contain specific binding sites for NHCR may also be

generated. For example, such fragments include, but are not limited to, the F(ab')2 fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse, W.D. et al. (1989) Science 254:1275-1281).

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between NHCR and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering NHCR epitopes is preferred, but a competitive binding assay may also be employed (Maddox, supra).

In another embodiment of the invention, the polynucleotides encoding NHCR, or any fragment thereof, or antisense molecules, may be used for therapeutic purposes. In one aspect, antisense to the polynucleotide encoding NHCR may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding NHCR. Thus, antisense molecules may be used to modulate NHCR activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligomers or larger fragments, can be designed from various locations along the coding or control regions of sequences encoding NHCR.

Expression vectors derived from retro viruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods which are well known to those skilled in the art can be used to construct recombinant vectors which will express antisense molecules complementary to the polynucleotides of the gene encoding NHCR. These techniques are described both in Sambrook et al. (supra) and in Ausubel et al. (supra).

Genes encoding NHCR can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide or fragment thereof which encodes NHCR. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements

are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing antisense molecules, DNA, RNA, or PNA, to the control regions of the gene encoding NHCR, i.e., the promoters, enhancers, and introns. Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J.E. et al. (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY). The antisense molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples which may be used include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding NHCR.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Antisense molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding NHCR. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible

modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection and by liposome injections may be achieved using methods which are well known in the art.

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of NHCR, antibodies to NHCR, mimetics, agonists, antagonists, or inhibitors of NHCR. The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton,

PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or

triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of NHCR, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example NHCR or fragments thereof, antibodies of NHCR, agonists, antagonists or inhibitors of NHCR, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which

exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind NHCR may be used for the diagnosis of conditions or diseases characterized by expression of NHCR, or in assays to monitor patients being treated with NHCR, agonists, antagonists or inhibitors. The antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for NHCR include methods which utilize the antibody and a label to detect NHCR in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules which are known in the art may be used, several of which are described above.

A variety of protocols including ELISA, RIA, and FACS for measuring NHCR are known in the art and provide a basis for diagnosing altered or abnormal levels of NHCR expression. Normal or standard values for NHCR expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to NHCR

under conditions suitable for complex formation The amount of standard complex formation may be quantified by various methods, but preferably by photometric, means. Quantities of NHCR expressed in subject, control and disease, samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding NHCR may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, antisense RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of NHCR may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess expression of NHCR, and to monitor regulation of NHCR levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding NHCR or closely related molecules, may be used to identify nucleic acid sequences which encode NHCR. The specificity of the probe, whether it is made from a highly specific region, e.g., 10 unique nucleotides in the 5' regulatory region, or a less specific region, e.g., especially in the 3' coding region, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low) will determine whether the probe identifies only naturally occurring sequences encoding NHCR, alleles, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably contain at least 50% of the nucleotides from any of the NHCR encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and derived from the nucleotide sequence of SEQ ID NO:2 or from genomic sequence including promoter, enhancer elements, and introns of the naturally occurring NHCR.

Means for producing specific hybridization probes for DNAs encoding NHCR include the cloning of nucleic acid sequences encoding NHCR or NHCR derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, radionuclides such as 32P or 35S, or enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding NHCR may be used for the diagnosis of disorders which are associated with expression of NHCR. Examples of such disorders may include, but are not limited to; allergic reactions, asthma and adult respiratory distress syndrome, inflammatory disorders such as rheumatoid arthritis, osteoarthritis, glomerulonephritis, osteoporosis, dermatomyositis, polymyositis, Addison's disease, Grave's disease, irritable bowel syndrome, atrophic gastritis, lupus erythematosus, myasthenia gravis, multiple sclerosis, autoimmune thyroiditis, ulcerative colitis, anemia, pancreatitis, scleroderma, Crohn's disease, ischemia/reperfusion injury, post-traumatic inflammation, myocardial inflammation, atherosclerosis, multiple sclerosis, HIV infection, Epstein-Barr virus, VZV, HTLV, CMV, bunyaviruses, influenza, adenovirus, rhinoviruses, hepadnaviruses, and rotaviruses sepsis, local tumor destruction, tumor regression, tissue repair; and inflammatory complications of cancer, hemodialysis and extracorporeal circulation, infection and trauma. The polynucleotide sequences encoding NHCR may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; or in dip stick, pin, ELISA or chip assays utilizing fluids or tissues from patient biopsies to detect altered NHCR expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding NHCR may be useful in assays that detect activation or induction of various cancers, particularly those mentioned above. The nucleotide sequences encoding NHCR may be labeled by standard methods, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the biopsied or extracted sample is significantly altered from that of a comparable control sample, the nucleotide sequences have hybridized with nucleotide sequences in the sample, and the presence of altered levels of nucleotide sequences encoding NHCR in the sample indicates the presence of the associated disease. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

In order to provide a basis for the diagnosis of disease associated with expression of NHCR, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, which encodes NHCR, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the

values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease.

Once disease is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that which is observed in the normal patient. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding NHCR may involve the use of PCR. Such oligomers may be chemically synthesized, generated enzymatically, or produced from a recombinant source. Oligomers will preferably consist of two nucleotide sequences, one with sense orientation (5'->3') and another with antisense (3'<-5'), employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of NHCR include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated (Melby, P.C. et al. (1993) J. Immunol. Methods, 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 229-236). The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In another embodiment of the invention, the nucleic acid sequences which encode NHCR may also be used to generate hybridization probes which are useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome or to a

specific region of the chromosome using well known techniques. Such techniques include FISH, FACS, or artificial chromosome constructions, such as yeast artificial chromosomes, bacterial artificial chromosomes, bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price, C.M. (1993) Blood Rev. 7:127-134, and Trask, B.J. (1991) Trends Genet. 7:149-154.

FISH (as described in Verma et al. (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY) may be correlated with other physical chromosome mapping techniques and genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of the gene encoding NHCR on a physical chromosomal map and a specific disease , or predisposition to a specific disease, may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier, or affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti, R.A. et al. (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

In another embodiment of the invention, NHCR, its catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes, between NHCR and the agent being tested, may be measured.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564. In this method, as applied to NHCR large numbers of

different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with NHCR, or fragments thereof, and washed. Bound NHCR is then detected by methods well known in the art. Purified NHCR can also be coated directly onto plates for use in the aforementioned drug screening techniques.

Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding NHCR specifically compete with a test compound for binding NHCR. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with NHCR.

In additional embodiments, the nucleotide sequences which encode NHCR may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

INDUSTRIAL APPLICABILITY

I MMLR3DT01 cDNA Library Construction

The normal peripheral blood macrophages used for this library were obtained from two 24 year old, Caucasian males. This library represents a mixture of allogeneically stimulated human macrophage populations obtained from Ficoll/Hypaque purified buffy coats. The cells from the two different donors (not typed for HLA alleles) were incubated at a density of 1 x 10^6/ml for 72 hours in DME containing 10% human serum.

After incubation, macrophages mostly adhered to the plastic surface of the petri dish, whereas most other cell types, B and T lymphocytes, remained in solution. The DME was decanted from the wells, and the wells were washed with phosphate buffered saline (PBS). Macrophages were released from the plastic surface by gently scraping the petri dishes in PBS/1 mM EDTA and lysed immediately in buffer containing guanidinium isothiocyanate.

The lysate was extracted twice with a mixture of acid phenol pH 4.0 and centrifuged over a CsCl cushion using an Beckman SW28 rotor in a L8-70M Ultracentrifuge (Beckman Instruments). The RNA was precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in water and DNase treated for 15 min at 37°C. It must be noted that some contaminating T and B lymphocytes may have been present.

The RNA was used in the SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning (catalogue #18248-013; Gibco BRL, Gaithersburg MD) with the recommended protocol. cDNAs were fractionated on a Sepharose CL4B column (catalog #275105, Pharmacia), and those cDNAs exceeding 400 bp were ligated into pSport I. The plasmid was transformed into chemically competent DH5α host cells (Gibco BRL).

II Isolation and Sequencing of cDNA Clones

Plasmid DNA was released from the cells and purified using the Miniprep Kit (Catalogue # 77468; Advanced Genetic Technologies Corporation, Gaithersburg MD). This kit consists of a 96 well block with reagents for 960 purifications. The recommended protocol was employed except for the following changes: 1) the 96 wells were each filled with only 1 ml of sterile Terrific Broth (Catalog # 22711, Gibco/BRL, Gaithersburg MD) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) the bacteria were cultured for 24 hours after the wells were inoculated and then lysed with 60 μl of lysis buffer; 3) a centrifugation step employing the Beckman GS-6R @2900 rpm for 5 min was performed before the contents of the block were added to the primary filter plate; and 4) the optional step of adding isopropanol to TRIS buffer was not routinely performed. After the last step in the protocol, samples were transferred to a Beckman 96-well block for storage.

The cDNAs were sequenced by the method of Sanger F and AR Coulson (1975; J Mol Biol 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown MA) and Applied Biosystems 377 or 373 DNA Sequencing Systems (Perkin Elmer) and reading frame was determined.

III Homology Searching of cDNA Clones and Their Deduced Proteins

Each cDNA was compared to sequences in GenBank using a search algorithm developed by Applied Biosystems and incorporated into the INHERIT™ 670 sequence analysis system. In this algorithm, Pattern Specification Language (TRW Inc, Los Angeles, CA) was used to determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database was searched for sequences containing regions of homology to the query sequence, and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman alignments were used to display the results of the homology search.

Peptide and protein sequence homologies were ascertained using the INHERIT- 670

sequence analysis system using the methods similar to those used in DNA sequence homologies. Pattern Specification Language and parameter windows were used to search protein databases for sequences containing regions of homology which were scored with an initial value. Dot-matrix homology plots were examined to distinguish regions of significant homology from chance matches.

BLAST, which stands for Basic Local Alignment Search Tool (Altschul, S.F. (1993) J. Mol. Evol. 36:290-300; Altschul et al. (1990) J. Mol. Biol. 215:403-410), was used to search for local sequence alignments. BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs. BLAST is useful for matches which do not contain gaps. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).

An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output.

IV Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound (Sambrook et al., supra).

Analogous computer techniques using BLAST (Altschul, S.F. 1993 and 1990, supra) are used to search for identical or related molecules in nucleotide databases such as GenBank or the LIFESEQ™ database (Incyte Pharmaceuticals). This analysis is much faster than multiple, membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

The basis of the search is the product score which is defined as:

$$\frac{\%\ \text{sequence identity} \times \%\ \text{maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1-2% error; and at 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analysis are reported as a list of libraries in which the transcript encoding NHCR occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

V Extension of NHCR-Encoding Polynucleotides

Nucleic acid sequence of Incyte clone 568987 or SEQ ID NO:2 is used to design oligonucleotide primers for extending a partial nucleotide sequence to full length or for obtaining 5' or 3', intron or other control sequences from genomic libraries. One primer is synthesized to initiate extension in the antisense direction (XLR) and the other is synthesized to extend sequence in the sense direction (XLF). Primers are used to facilitate the extension of the known sequence "outward" generating amplicons containing new, unknown nucleotide sequence for the region of interest. The initial primers are designed from the cDNA using OLIGO 4.06 (National Biosciences), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations is avoided.

The original, selected cDNA libraries, or a human genomic library are used to extend the sequence; the latter is most useful to obtain 5' upstream regions. If more extension is necessary or desired, additional sets of primers are designed to further extend the known region.

By following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix, high fidelity amplification is obtained. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, PCR is performed using the Peltier Thermal Cycler (PTC200; M.J. Research, Watertown, MA) and the following parameters:

Step 1	94° C for 1 min (initial denaturation)
Step 2	65° C for 1 min
Step 3	68° C for 6 min
Step 4	94° C for 15 sec

Step 5	65° C for 1 min
Step 6	68° C for 7 min
Step 7	Repeat step 4-6 for 15 additional cycles
Step 8	94° C for 15 sec
Step 9	65° C for 1 min
Step 10	68° C for 7:15 min
Step 11	Repeat step 8-10 for 12 cycles
Step 12	72° C for 8 min
Step 13	4° C (and holding)

A 5-10 μl aliquot of the reaction mixture is analyzed by electrophoresis on a low concentration (about 0.6-0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products are selected and removed from the gel. Further purification involves using a commercial gel extraction method such as QIAQuick™ (QIAGEN Inc., Chatsworth, CA). After recovery of the DNA, Klenow enzyme is used to trim single-stranded, nucleotide overhangs creating blunt ends which facilitate religation and cloning.

After ethanol precipitation, the products are redissolved in 13 μl of ligation buffer, 1μl T4-DNA ligase (15 units) and 1μl T4 polynucleotide kinase are added, and the mixture is incubated at room temperature for 2-3 hours or overnight at 16° C. Competent E. coli cells (in 40 μl of appropriate media) are transformed with 3 μl of ligation mixture and cultured in 80 μl of SOC medium (Sambrook et al., supra). After incubation for one hour at 37° C, the whole transformation mixture is plated on Luria Bertani (LB)-agar (Sambrook et al., supra) containing 2x Carb. The following day, several colonies are randomly picked from each plate and cultured in 150 μl of liquid LB/2x Carb medium placed in an individual well of an appropriate, commercially-available, sterile 96-well microtiter plate. The following day, 5 μl of each overnight culture is transferred into a non-sterile 96-well plate and after dilution 1:10 with water, 5 μl of each sample is transferred into a PCR array.

For PCR amplification, 18 μl of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction are added to each well. Amplification is performed using the following conditions:

Step 1	94° C for 60 sec
Step 2	94° C for 20 sec
Step 3	55° C for 30 sec
Step 4	72° C for 90 sec
Step 5	Repeat steps 2-4 for an additional 29 cycles
Step 6	72° C for 180 sec

Step 7 4° C (and holding)

Aliquots of the PCR reactions are run on agarose gels together with molecular weight markers. The sizes of the PCR products are compared to the original partial cDNAs, and appropriate clones are selected, ligated into plasmid, and sequenced.

VI Labeling and Use of Hybridization Probes

Hybridization probes derived from SEQ ID NO:2 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences), labeled by combining 50 pmol of each oligomer and 250 μCi of [γ-^{32}P] adenosine triphosphate (Amersham) and T4 polynucleotide kinase (DuPont NEN®, Boston, MA). The labeled oligonucleotides are substantially purified with Sephadex G-25 superfine resin column (Pharmacia & Upjohn). A portion containing 10^7 counts per minute of each of the sense and antisense oligonucleotides is used in a typical membrane based hybridization analysis of human genomic DNA digested with one of the following endonucleases (Ase I, Bgl II, Eco RI, Pst I, Xba 1, or Pvu II; DuPont NEN®).

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR™ film (Kodak, Rochester, NY) is exposed to the blots in a Phosphoimager cassette (Molecular Dynamics, Sunnyvale, CA) for several hours, hybridization patterns are compared visually.

VII Antisense Molecules

Antisense molecules to the NHCR-encoding sequence, or any part thereof, is used to inhibit in vivo or in vitro expression of naturally occurring NHCR. Although use of antisense oligonucleotides, comprising about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. An oligonucleotide based on the coding sequences of NHCR, as shown in Figures 1A, 1B, 1C, 1D and 1E, is used to inhibit expression of naturally occurring NHCR. The complementary oligonucleotide is designed from the most unique 5' sequence as shown in Figures 1A, 1B, 1C, 1D and 1E and used either to inhibit transcription by preventing promoter binding to the upstream nontranslated sequence or translation of an NHCR-encoding transcript by preventing the ribosome from binding. Using an

appropriate portion of the signal and 5' sequence of SEQ ID NO:2, an effective antisense oligonucleotide includes any 15-20 nucleotides spanning the region which translates into the signal or 5' coding sequence of the polypeptide as shown in Figures 1A, 1B, 1C, 1D and 1E.

VIII Expression of NHCR

Expression of NHCR is accomplished by subcloning the cDNAs into appropriate vectors and transforming the vectors into host cells. In this case, the cloning vector, pSport, previously used for the generation of the cDNA library is used to express NHCR in E. coli. Upstream of the cloning site, this vector contains a promoter for ß-galactosidase, followed by sequence containing the amino-terminal Met, and the subsequent seven residues of ß-galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker containing a number of unique restriction sites.

Induction of an isolated, transformed bacterial strain with IPTG using standard methods produces a fusion protein which consists of the first eight residues of ß-galactosidase, about 5 to 15 residues of linker, and the full length protein. The signal residues direct the secretion of NHCR into the bacterial growth media which can be used directly in the following assay for activity.

IX Demonstration of NHCR Activity

GTP-binding activity is assayed by incubating varying amounts of NHCR protein for 10 minutes at 30°C in 50mM Tris buffer, pH 7.5, containing 1mM dithiothreitol, 1mM EDTA, 1μM (a-^{32}P), in the absence or presence of 100μM of the following compounds: GTP, GDP, GTPγS, ATP, CTP, UTP, and TTP. Samples are passed through nitrocellulose filters and washed twice with a buffer consisting of 50mM Tris-HCL, pH 7.8, 1mM NaN_3, 10mM $MgCl_2$, 1mM EDTA, 0.5mM dithiothreitol, 0.01mM PMSF, and 200mM NaCl. The filter-bound counts are determined by liquid scintillation. To determine GTPase activity, BND protein is incubated for 10 minutes at 37°C in 50mM Tris-HCL buffer, pH 7.8, containing 1mM dithiothreitol, 2mM EDTA, 10μM (a-^{32}P), and 1μM H-rab protein. GTPase activity is initiated by adding $MgCl_2$ to a final concentration of 10 mM. Samples are removed at various time points, mixed with an equal volume of ice-cold 0.5mM EDTA, and frozen. Aliquots are spotted onto polyethyleneimine-cellulose thin layer chromatography plates, which are developed in 1M LiCl, dried, and autoradiographed.

X Production of NHCR Specific Antibodies

NHCR that is substantially purified using PAGE electrophoresis (Sambrook, supra), or other purification techniques, is used to immunize rabbits and to produce antibodies using

standard protocols. The amino acid sequence deduced from SEQ ID NO:2 is analyzed using DNASTAR software (DNASTAR Inc) to determine regions of high immunogenicity and a corresponding oligopolypeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions, is described by Ausubel et al. (supra), and others.

Typically, the oligopeptides are 15 residues in length, synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry, and coupled to keyhole limpet hemocyanin (KLH, Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel et al., supra). Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radioiodinated, goat anti-rabbit IgG.

XI Purification of Naturally Occurring NHCR Using Specific Antibodies

Naturally occurring or recombinant NHCR is substantially purified by immunoaffinity chromatography using antibodies specific for NHCR. An immunoaffinity column is constructed by covalently coupling NHCR antibody to an activated chromatographic resin, such as CnBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing NHCR is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of NHCR (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/NHCR binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope, such as urea or thiocyanate ion), and NHCR is collected.

XII Identification of Molecules Which Interact with NHCR

NHCR or biologically active fragments thereof are labeled with ^{125}I Bolton-Hunter reagent (Bolton et al. (1973) Biochem. J. 133: 529). Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled NHCR, washed and any wells with labeled NHCR complex are assayed. Data obtained using different concentrations of NHCR are used to calculate values for the number, affinity, and association of NHCR with the candidate molecules.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit

of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: INCYTE PHARMACEUTICALS, INC.

(ii) TITLE OF THE INVENTION: NOVEL HUMAN CHEMOKINE RECEPTOR-LIKE PROTEIN

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Incyte Pharmaceuticals, Inc.
(B) STREET: 3174 Porter Dr.
(C) CITY: Palo Alto
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94304

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) PCT APPLICATION NUMBER:To Be Assigned
(B) FILING DATE:Filed Herewith

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/812,871
(B) FILING DATE: 06-MAR-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Billings, Lucy J.
(B) REGISTRATION NUMBER: 36,749
(C) REFERENCE/DOCKET NUMBER: PF-0237 PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650-855-0555
(B) TELEFAX: 650-845-4166

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 333 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(vii) IMMEDIATE SOURCE:
(A) LIBRARY: MMLR3DT01
(B) CLONE: 568987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Asn Thr Thr Val Met Gln Gly Phe Asn Arg Ser Glu Arg Cys Pro
1 5 10 15

```
Arg Asp Thr Arg Ile Val Gln Leu Val Phe Pro Ala Leu Tyr Thr Val
            20                  25                  30
Val Phe Leu Thr Gly Ile Leu Leu Asn Thr Leu Ala Leu Trp Val Phe
        35                  40                  45
Val His Ile Pro Ser Ser Ser Thr Phe Ile Ile Tyr Leu Lys Asn Thr
    50                  55                  60
Leu Val Ala Asp Leu Ile Met Thr Leu Met Leu Pro Phe Lys Ile Leu
65                  70                  75                  80
Ser Asp Ser His Leu Ala Pro Trp Gln Leu Arg Ala Phe Val Cys Arg
                85                  90                  95
Phe Ser Ser Val Ile Phe Tyr Glu Thr Met Tyr Val Gly Ile Val Leu
            100                 105                 110
Leu Gly Leu Ile Ala Phe Asp Arg Phe Leu Lys Ile Ile Arg Pro Leu
        115                 120                 125
Arg Asn Ile Phe Leu Lys Lys Pro Val Phe Ala Lys Thr Val Ser Ile
    130                 135                 140
Phe Ile Trp Phe Phe Leu Phe Phe Ile Ser Leu Pro Ile Met Ile Leu
145                 150                 155                 160
Ser Asn Lys Glu Ala Thr Pro Ser Ser Val Lys Lys Cys Ala Ser Leu
                165                 170                 175
Lys Gly Pro Leu Gly Leu Lys Trp His Gln Met Val Asn Asn Ile Cys
            180                 185                 190
Gln Phe Ile Phe Trp Thr Val Leu Ile Leu Met Leu Val Phe Tyr Val
        195                 200                 205
Val Ile Ala Lys Lys Val Tyr Asp Ser Tyr Arg Lys Ser Lys Cys Lys
    210                 215                 220
Asp Arg Lys Asn Asn Lys Lys Leu Glu Gly Lys Val Phe Val Val Val
225                 230                 235                 240
Pro Val Phe Phe Val Cys Phe Ala Pro Phe His Phe Ala Arg Val Pro
                245                 250                 255
Tyr Thr His Ser Gln Thr Asn Asn Lys Thr Asp Cys Arg Leu Gln Asn
            260                 265                 270
Gln Leu Phe Ile Ala Lys Glu Thr Thr Leu Phe Leu Ala Ala Thr Asn
        275                 280                 285
Ile Cys Met Asp Pro Leu Ile Ser Ile Phe Leu Cys Lys Lys Phe Thr
    290                 295                 300
Glu Lys Leu Pro Cys Met Gln Gly Arg Lys Thr Thr Ala Ser Ser Gln
305                 310                 315                 320
Glu Asn His Ser Ser Gln Thr Asp Asn Ile Thr Leu Gly
                325                 330
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1488 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(A) LIBRARY: MMLR3DT01
(B) CLONE: 568987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
ACTAGTTCAA GAGGCCATCT ACGAACGTAT GACTGCCGCT TTAAGAAGAC AGAGAGAACT     60
GAGTATCCTC CCAAAGGTGA CACTGGAAGC AATGAACACC ACAGTGATGC AAGGCTTCAA    120
CAGATCTGAG CGGTGCCCCA GAGACACTCG GATAGTACAG CTGGTATTCC CAGCCCTCTA    180
CACAGTGGTT TTCTTGACCG GCATCCTGCT GAATACTTTG GCTCTGTGGG TGTTTGTTCA    240
CATCCCCAGC TCCTCCACCT TCATCATCTA CCTCAAAAAC ACTTTGGTGG CCGACTTGAT    300
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AATGACACTC ATGCTTCCTT TCAAAATCCT CTCTGACTCA CACCTGGCAC CCTGGCAGCT    360
CAGAGCTTTT GTGTGTCGTT TTTCTTCGGT GATATTTTAT GAGACCATGT ATGTGGGCAT    420
CGTGCTGTTA GGGCTCATAG CCTTTGACAG ATTCCTCAAG ATCATCAGAC CTTTGAGAAA    480
TATTTTTCTA AAAAAACCTG TTTTTGCAAA AACGGTCTCA ATCTTCATCT GGTTCTTTTT    540
GTTCTTCATC TCCCTGCCAA TTATGATCTT GAGCAACAAG GAAGCAACAC CATCGTCTGT    600
GAAAAAGTGT GCTTCCTTAA AGGGGCCTCT GGGGCTGAAA TGGCATCAAA TGGTAAATAA    660
CATATGCCAG TTTATTTTCT GGACTGTTTT AATCCTAATG CTTGTGTTTT ATGTGGTTAT    720
TGCAAAAAAA GTATATGATT CTTATAGAAA GTCCAAATGT AAGGACAGAA AAAACAACAA    780
AAAGCTGGAA GGCAAAGTAT TTGTTGTCGT GCCTGTCTTC TTTGTGTGTT TTGCTCCATT    840
TCATTTTGCC AGAGTTCCAT ATACTCACAG TCAAACCAAC AATAAGACTG ACTGTAGACT    900
GCAAAATCAA CTGTTTATTG CTAAAGAAAC AACTCTCTTT TTGGCAGCAA CTAACATTTG    960
TATGGATCCC TTAATATCCA TATTCTTATG TAAAAAATTC ACAGAAAAGC TACCATGTAT   1020
GCAAGGGAGA AAGACCACAG CATCAAGCCA AGAAAATCAT AGCAGTCAGA CAGACAACAT   1080
AACCTTAGGC TGACAACTGT ACATAGGGTT AACTTCTATT TATTGATGAG ACTTCCGTAG   1140
ATAATGTGGA AATCAAATTT AACCAAGAAA AAAAGATTGG AACAAATGCT CTCTTACATT   1200
TTATTATCCT GGTGTACAGA AAAGATTATA TAAAATTTAA ATCCACATAG ATCTATTCAT   1260
AAGCTGAATG AACCATTACT AAGAGAATGC AACAGGATAC AAATGGCCAC TAGAGGTCAT   1320
TATTTCTTTC TTTCTTTTTT TTTTTTTTTA ATTTCAAGAG CATTTCACTT TAACATTTTG   1380
GAAAAGACTA AGGAGAAACG TATATCCCTA CAAACCTCCC CTCTAAACAC CTTCTCACAT   1440
TTTTTTCCAC AATTCACATA ACACTACTGC TTTTGTCCCC TTAAATGT                1488
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: GenBank
(B) CLONE: 992700

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Asn Gly Leu Glu Val Ala Pro Pro Gly Leu Ile Thr Asn Phe Ser
 1               5                  10                  15
Leu Ala Thr Ala Glu Gln Cys Gly Gln Glu Thr Pro Leu Glu Asn Met
            20                  25                  30
Leu Phe Ala Ser Phe Tyr Leu Leu Asp Phe Ile Leu Ala Leu Val Gly
        35                  40                  45
Asn Thr Leu Ala Leu Trp Leu Phe Ile Arg Asp His Lys Ser Gly Thr
    50                  55                  60
Pro Ala Asn Val Phe Leu Met His Leu Ala Val Ala Asp Leu Ser Cys
65                  70                  75                  80
Val Leu Val Leu Pro Thr Arg Leu Val Tyr His Phe Ser Gly Asn His
                85                  90                  95
Trp Pro Phe Gly Glu Ile Ala Cys Arg Leu Thr Gly Phe Leu Phe Tyr
            100                 105                 110
Leu Asn Met Tyr Ala Ser Ile Tyr Phe Leu Thr Cys Ile Ser Ala Asp
        115                 120                 125
Arg Phe Leu Ala Ile Val His Pro Val Lys Ser Leu Lys Leu Arg Arg
    130                 135                 140
Pro Leu Tyr Ala His Leu Ala Cys Ala Phe Leu Trp Val Val Val Ala
145                 150                 155                 160
Val Ala Met Ala Pro Leu Leu Val Ser Pro Gln Thr Val Gln Thr Asn
                165                 170                 175
His Thr Val Val Cys Leu Gln Leu Tyr Arg Glu Lys Ala Ser His His
            180                 185                 190
```

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Ala Leu Val Ser Leu Ala Val Ala Phe Thr Phe Pro Phe Ile Thr Thr
        195                 200                 205
Val Thr Cys Tyr Leu Leu Ile Ile Arg Ser Leu Arg Gln Gly Leu Arg
    210                 215                 220
Val Glu Lys Arg Leu Lys Thr Lys Ala Val Arg Met Ile Ala Ile Val
225                 230                 235                 240
Leu Ala Ile Phe Leu Val Cys Phe Val Pro Tyr His Val Asn Arg Ser
                245                 250                 255
Val Tyr Val Leu His Tyr Arg Ser His Gly Ala Ser Cys Ala Thr Gln
            260                 265                 270
Arg Ile Leu Ala Leu Ala Asn Arg Ile Thr Ser Cys Leu Thr Ser Leu
        275                 280                 285
Asn Gly Ala Leu Asp Pro Ile Met Tyr Phe Phe Val Ala Glu Lys Phe
    290                 295                 300
Arg His Ala Leu Cys Asn Leu Leu Cys Gly Lys Arg Leu Lys Gly Pro
305                 310                 315                 320
Pro Pro Ser Phe Glu Gly Lys Thr Asn Glu Ser Ser Leu Ser Ala Lys
                325                 330                 335
Ser Glu Leu
```

What is claimed is:

1. A substantially purified human chemokine receptor-like protein (NHCR) comprising the amino acid sequence of SEQ ID NO:1 or fragments thereof.

2. An isolated and purified polynucleotide sequence encoding the human chemokine receptor-like protein of claim 1.

3. A polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence of claim 2.

4. A hybridization probe comprising the polynucleotide sequence of claim 2.

5. An isolated and purified polynucleotide sequence comprising SEQ ID NO:2 or variants thereof.

6. A polynucleotide sequence which is complementary to the polynucleotide sequence of claim 2 or variants thereof.

7. A hybridization probe comprising the polynucleotide sequence of claim 6.

8. An expression vector containing the polynucleotide sequence of claim 2.

9. A host cell containing the vector of claim 8.

10. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1 the method comprising the steps of:

a) culturing the host cell of claim 9 under conditions suitable for the expression of the polypeptide; and

b) recovering the polypeptide from the host cell culture.

11. A pharmaceutical composition comprising a substantially purified human chemokine receptor-like protein having an amino acid sequence of claim 1 in conjunction with a suitable pharmaceutical carrier.

12. A purified antibody which binds specifically to the polypeptide of claim 1.

13. A purified agonist which modulates the activity of the polypeptide of claim 1.

14. A purified antagonist which inhibits the activity of the polypeptide of claim 1.

15. A method for treating a disorder which is associated with decreased expression of human chemokine receptor-like protein comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 11.

16. A pharmaceutical composition comprising the antagonist of claim 14 in conjunction with a suitable pharmaceutical carrier.

17. A method for treating an inflammatory disorder comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim

16.

18. A method for treating a viral infection which is associated with expression of human chemokine receptor-like protein comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 16.

19. A method for detection of a polynucleotide encoding human chemokine receptor-like protein in a biological sample comprising the steps of:

a) hybridizing the polynucleotide of claim 6 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex, wherein the presence of said complex correlates with the presence of a polynucleotide encoding human chemokine receptor-like protein in said biological sample.

```
          9          18          27          36          45          54
CTA GTT CAA GAG GCC ATC TAC GAA CGT ATG ACT GCC GCT TTA AGA AGA CAG AGA

         63          72          81          90          99         108
GAA CTG AGT ATC CTC CCA AAG GTG ACA CTG GAA GCA ATG AAC ACC ACA GTG ATG
                                                M   N   T   T   V   M

        117         126         135         144         153         162
CAA GGC TTC AAC AGA TCT GAG CGG TGC CCC AGA GAC ACT CGG ATA GTA CAG CTG
Q   G   F   N   R   S   E   R   C   P   R   D   T   R   I   V   Q   L

        171         180         189         198         207         216
GTA TTC CCA GCC CTC TAC ACA GTG GTT TTC TTG ACC GGC ATC CTG CTG AAT ACT
V   F   P   A   L   Y   T   V   V   F   L   T   G   I   L   L   N   T

        225         234         243         252         261         270
TTG GCT CTG TGG GTG TTT GTT CAC ATC CCC AGC TCC TCC ACC TTC ATC ATC TAC
L   A   L   W   V   F   V   H   I   P   S   S   S   T   F   I   I   Y

        279         288         297         306         315         324
CTC AAA AAC ACT TTG GTG GCC GAC TTG ATA ATG ACA CTC ATG CTT CCT TTC AAA
L   K   N   T   L   V   A   D   L   I   M   T   L   M   L   P   F   K
```

FIGURE 1A

```
         333        342        351        360        369        378
ATC CTC TCT GAC TCA CAC CTG GCA CCC TGG CAG CTC AGA GCT TTT GTG TGT CGT
I   L   S   D   S   H   L   A   P   W   Q   L   R   A   F   V   C   R

         387        396        405        414        423        432
TTT TCT TCG GTG ATA TTT TAT GAG ACC ATG TAT GTG GGC ATC GTG CTG TTA GGG
F   S   S   V   I   F   Y   E   T   M   Y   V   G   I   V   L   L   G

         441        450        459        468        477        486
CTC ATA GCC TTT GAC AGA TTC CTC AAG ATC ATC AGA CCT TTG AGA AAT ATT TTT
L   I   A   F   D   R   F   L   K   I   I   R   P   L   R   N   I   F

         495        504        513        522        531        540
CTA AAA AAA CCT GTT TTT GCA AAA ACG GTC TCA ATC TTC ATC TGG TTC TTT TTG
L   K   K   P   V   F   A   K   T   V   S   I   F   I   W   F   F   L

         549        558        567        576        585        594
TTC TTC ATC TCC CTG CCA ATT ATG ATC TTG AGC AAC AAG GAA GCA ACA CCA TCG
F   F   I   S   L   P   I   M   I   L   S   N   K   E   A   T   P   S

         603        612        621        630        639        648
TCT GTG AAA AAG TGT GCT TCC TTA AAG GGG CCT CTG GGG CTG AAA TGG CAT CAA
S   V   K   K   C   A   S   L   K   G   P   L   G   L   K   W   H   Q
```

FIGURE 1B

```
          657        666        675        684        693        702
ATG GTA AAT AAC ATA TGC CAG TTT ATT TTC TGG ACT GTT TTA ATC CTA ATG CTT
M   V   N   N   I   C   Q   F   I   F   W   T   V   L   I   L   M   L

          711        720        729        738        747        756
GTG TTT TAT GTG GTT ATT GCA AAA AAA GTA TAT GAT TCT TAT AGA AAG TCC AAA
V   F   Y   V   V   I   A   K   K   V   Y   D   S   Y   R   K   S   K

          765        774        783        792        801        810
TGT AAG GAC AGA AAA AAC AAC AAA AAG CTG GAA GGC AAA GTA TTT GTT GTC GTG
C   K   D   R   K   N   N   K   K   L   E   G   K   V   F   V   V   V

          819        828        837        846        855        864
CCT GTC TTC TTT GTG TGT TTT GCT CCA TTT CAT TTT GCC AGA GTT CCA TAT ACT
P   V   F   F   V   C   F   A   P   F   H   F   A   R   V   P   Y   T

          873        882        891        900        909        918
CAC AGT CAA ACC AAC AAT AAG ACT GAC TGT AGA CTG CAA AAT CAA CTG TTT ATT
H   S   Q   T   N   N   K   T   D   C   R   L   Q   N   Q   L   F   I

          927        936        945        954        963        972
GCT AAA GAA ACA ACT CTC TTT TTG GCA GCA ACT AAC ATT TGT ATG GAT CCC TTA
A   K   E   T   T   L   F   L   A   A   T   N   I   C   M   D   P   L
```

FIGURE 1C

```
         981         990         999        1008        1017        1026
ATA TCC ATA TTC TTA TGT AAA AAA TTC ACA GAA AAG CTA CCA TGT ATG CAA GGG
I   S   I   F   L   C   K   K   F   T   E   K   L   P   C   M   Q   G

        1035        1044        1053        1062        1071        1080
AGA AAG ACC ACA GCA TCA AGC CAA GAA AAT CAT AGC AGT CAG ACA GAC AAC ATA
R   K   T   T   A   S   S   Q   E   N   H   S   S   Q   T   D   N   I

        1089        1098        1107        1116        1125        1134
ACC TTA GGC TGA CAA CTG TAC ATA GGG TTA ACT TCT ATT TAT TGA TGA GAC TTC
T   L   G

        1143        1152        1161        1170        1179        1188
CGT AGA TAA TGT GGA AAT CAA ATT TAA CCA AGA AAA AAA GAT TGG AAC AAA TGC

        1197        1206        1215        1224        1233        1242
TCT CTT ACA TTT TAT TAT CCT GGT GTA CAG AAA AGA TTA TAT AAA ATT TAA ATC

        1251        1260        1269        1278        1287        1296
CAC ATA GAT CTA TTC ATA AGC TGA ATG AAC CAT TAC TAA GAG AAT GCA ACA GGA
```

FIGURE 1D

```
      1305        1314        1323        1332        1341        1350
TAC AAA TGG CCA CTA GAG GTC ATT ATT TCT TTC TTT CTT TTT TTT TTT TTT TAA

      1359        1368        1377        1386        1395        1404
TTT CAA GAG CAT TTC ACT TTA ACA TTT TGG AAA AGA CTA AGG AGA AAC GTA TAT

      1413        1422        1431        1440        1449        1458
CCC TAC AAA CCT CCC CTC TAA ACA CCT TCT CAC ATT TTT TTC CAC AAT TCA CAT

      1467        1476        1485
AAC ACT ACT GCT TTT GTC CCC TTA AAT GT
```

FIGURE 1E

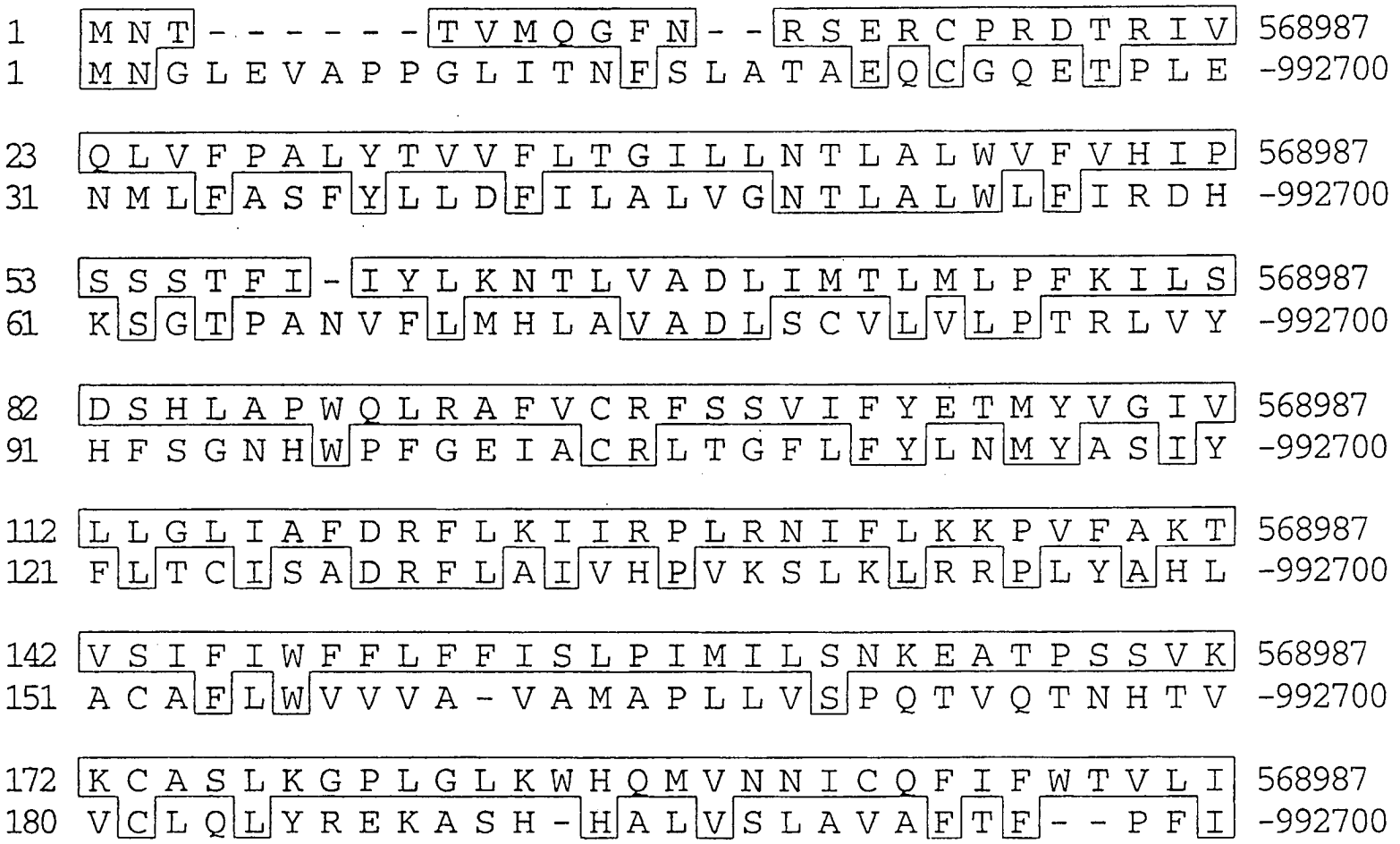
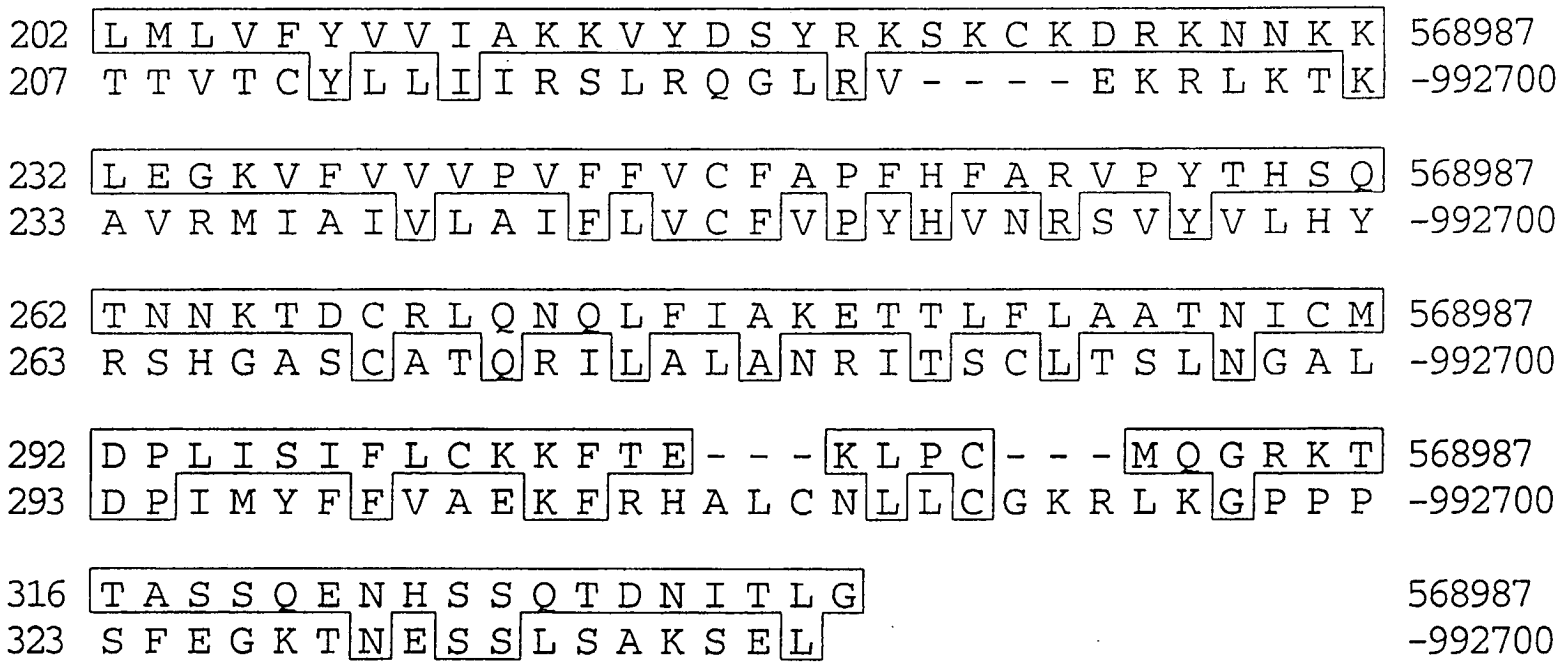

1 MNT-------TVMQGFN--RSERCPRDTRIV 568987
1 MNGLEVAPPGLITNFSLATAEQCGQETPLE -992700
23 QLVFPALYTVVFLTGILLNTLALWVFVHIP 568987
31 NMLFASFYLLDFILALVGNTLALWLFIRDH -992700
53 SSSTFI-IYLKNTLVADLIMTLMLPFKILS 568987
61 KSGTPANVFLMHLAVADLSCVLVLPTRLVY -992700
82 DSHLAPWQLRAFVCRFSSVIFYETMYVGIV 568987
91 HFSGNHWPFGEIACRLTGFLFYLNMYASIY -992700
112 LLGLIAFDRFLKIIRPLRNIFLKKPVFAKT 568987
121 FLTCISADRFLAIVHPVKSLKLRRPLYAHL -992700
142 VSIFIWFFLFFISLPIMILSNKEATPSSVK 568987
151 ACAFLWVVVA-VAMAPLLVSPQTVQTNHTV -992700
172 KCASLKGPLGLKWHQMVNNICQFIFWTVLI 568987
180 VCLQLYREKASH-HALVSLAVAFTF--PFI -992700

FIGURE 2A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207 T T V T C Y L L I I R S L R Q G L R V - - - - E K R L K T K -992700
232 L E G K V F V V V P V F F V C F A P F H F A R V P Y T H S Q 568987
233 A V R M I A I V L A I F L V C F V P Y H V N R S V Y V L H Y -992700
262 T N N K T D C R L Q N Q L F I A K E T T L F L A A T N I C M 568987
263 R S H G A S C A T Q R I L A L A N R I T S C L T S L N G A L -992700
292 D P L I S I F L C K K F T E - - - K L P C - - - M Q G R K T 568987
293 D P I M Y F F V A E K F R H A L C N L L C G K R L K G P P P -992700
316 T A S S Q E N H S S Q T D N I T L G 568987
323 S F E G K T N E S S L S A K S E L -992700

FIGURE 2B

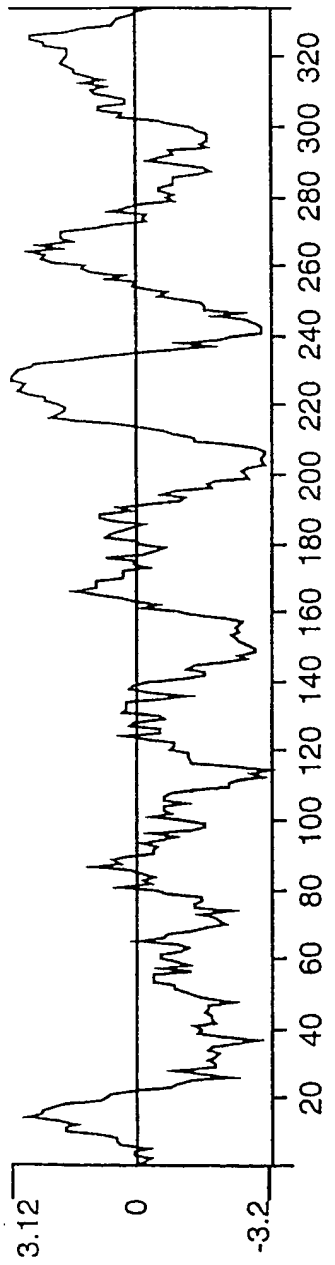

FIGURE 3A

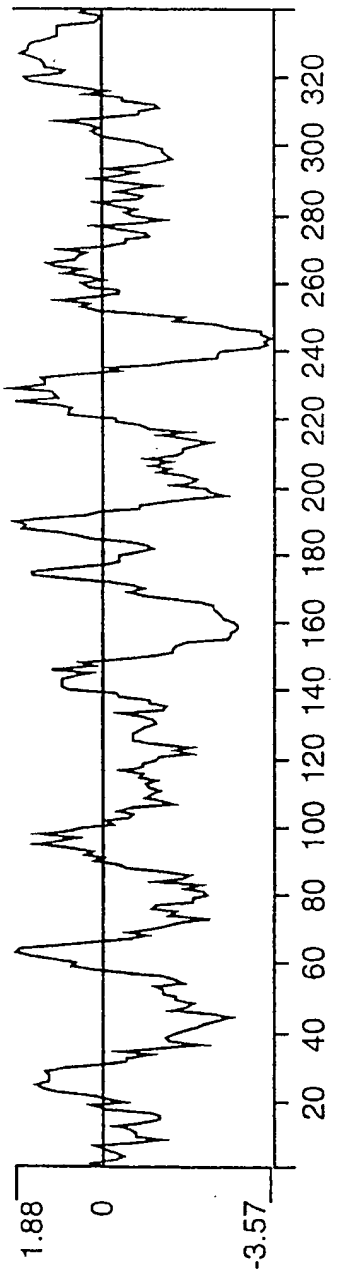

FIGURE 3B

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 98/04278

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/715 C07K16/28 A61K38/19 C12Q1/68
//C12N15/11,C12N9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 30406 A (HUMAN GENOME SCIENCES INC ;LI YI (US); CAO LIANG (GB); NI JIAN (US) 3 October 1996	1-16,19
Y	see the whole document, particularly where reference is made to GPR3.	17,18

Y	WO 96 23068 A (GLAXO GROUP LTD ;WELLS TIMOTHY NIGEL CARL (CH); POWER CHRISTINE AN) 1 August 1996 see the whole document	17,18

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 June 1998	06.07.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Smalt, R

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ational application No.
PCT/US 98/04278

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 15, 17 and 18 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

— Information on patent family members

Interna' ıl Application No
PCT/US 98/04278

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9630406 A	03-10-1996	AU 2236895 A	16-10-1996
		EP 0817800 A	14-01-1998
WO 9623068 A	01-08-1996	AU 4455896 A	14-08-1996
		EP 0805859 A	12-11-1997